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Pulse radiolysis of sparfloxacin in neutral aqueous solution

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Abstract A pulse radiolysis study was carried out to investigate the radical anion and radical cation of sparfloxacin (SPAX). The reactions of SPAX with hydrate electron (e_{aq}^-), hydroxyl radical ($\cdot$OH) and azide radical ($\cdot N_3^-$) were investigated in this study. The transient absorption spectra of SPAX radical anion and SPAX radical cation were obtained. Two transient rate constants 2.2×10^{10} $dm^3\cdot mol^{-1}\cdot s^{-1}$ and 1.7×10^{10} $dm^3\cdot mol^{-1}\cdot s^{-1}$ for the reactions of SPAX with e_{aq}^- and $\cdot$OH were determined, respectively. Finally, based on the results obtained in this study, rational mechanisms of transient reactions were proposed.

Key words Sparfloxacin, Pulse radiolysis, Radical cation, Radical anion

1 Introduction

Fluoroquinolones (FQs) antibiotics are often used to treat various bacterial infections by inhibiting bacterial DNA synthesis[1]. Sparfloxacin (SPAX) is an antibiotic drug from the fluoroquinolones family, which often used to treat bacterial urinary tract infections, sexually transmitted diseases, prostatitis, selected pneumonias, and skin infections[2–4]. SPAX could against a wide range of Gram-positive and Gram-negative organisms [5–8]. However, SPAX has a drawback in usage. It frequently elicits photosensitive skin reactions[9,10]. Report has suggested that SPAX was a phototoxic agent and can induce DNA stand break[11–14].

The most important target for radiation-induced reproductive cell death is generally considered to be the DNA of the cell[15–19]. Two components are believed to be responsible for DNA damage, the direct effect in the situation of ionizing radiation absorbed by the DNA itself, and the indirect effect in the situation of DNA attacked by active radicals, such as radical cation surrounding the DNA[15,20,21]. As stated above, the drug, SPAX can photo-induce DNA damage. Hence, it was reasonably to think that the SPAX oxidized radical may be an important factor inducing DNA damage.

Though sparfloxacin has been studied in terms of therapeutic activities[22–24], few reports about its transient products analysis are available in literatures published. Especially, no studies of SPAX radical cation and radical anion were found in papers. However, results of SPAX radical may be a good help to study the SPAX transient products and photo-induced DNA damage.

To investigate photochemical and photo-physical porosities of SPAX and the relationship between the structure and effects, pulse radiolysis studies of transient radical cation and radical anion were carried out in this study.

2 Materials and methods

Sparfloxacin was purchased from Sigma Chemical Co and Sodium azide was purchased from J&K Scientific Co, and they were all used without further purification. Water was purified by a Millipore Milli-Q system.

The nanosecond pulse radiolysis experiments were performed utilizing a 10 MeV linear accelerator, which delivers an electron pulse with duration of 8 ns.

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The dosimetry of electron pulse was determined by thiocyanate dosimeter using $G[(CNS)_2^{\cdot -}]$ = 5.8 in 0.1 mM KSCN saturated with N_2O by taking ε_{480} nm = 7600 $dm^3 \cdot mol^{-1} \cdot s^{-1}$ for $(SCN)_2^{\cdot -}$. The dose per electron pulse was 10 Gy. A Xenon lamp was employed as detecting light source. The electron pulse and the analyzing light beam passed perpendicularly through a 10 mm×10 mm×40 mm quartz cell. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The output signal from the LeCroy wavemaster 8600A digital oscillograph was transferred to a personal computer for further analysis. To create a reducing environment, tert-butanol (*t*-BuOH) was used to scavenge the ·OH and the sample solutions were saturated with N_2, hence e_{aq}^- remained. To create an almost uniform ·OH radical oxidizing solution environment, the sample solution was saturated with N_2O before pulse radiolysis, where e_{aq}^- is converted to ·OH, based on the reactions discribed below[25–28].

$$H_2O \rightarrow e_{aq}^-, H^\cdot, {}^\cdot OH, H_2, H_2O_2, H_3O^+$$

$${}^\cdot OH + \text{tert-BuOH} \rightarrow \text{tert-BuO} + H_2O,$$

$$K = 5.1\times10^8\ dm^3 \cdot mol^{-1} \cdot s^{-1}$$

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^\cdot OH + OH^- + N_2,$$

$$K = 8.7\times10^9\ dm^3 \cdot mol^{-1} \cdot s^{-1}$$

For the studies on ·OH with PAX, the sample solutions were saturated with N_2O to scavenge hydrated electrons e_{aq}^-.

3 Results and discussion

3.1 Reaction of SPAX with ·OH

Hydroxyl radical was a strong oxidizing agent and it was often used to study the radical cation or neutral radicals of target. In this study, the reaction of SPAX with hydroxyl radical was investigated. The transient absorption spectra obtained after pulse radiolysis of 0.1 mM SPAX neutral aqueous solution were shown in Fig.1. After pulse radiolysis, a strong inverted absorption at about 370 nm was observed, and it was assigned to a bleaching as the UV-Vis experiment result showed that SPAX has an maximum absorption at about 370 nm[29]. At the same time, a distinct transient absorption in the range of 400–550 nm with a maximum absorption at 420 nm appeared. As we all know that the reaction of hydroxyl radical with the target may be by three types: (1) hydrogen abstraction, (2) addition reaction and (3) one-electron oxidation[27,30,31]. Hence, it was reasonably to propose the following reactions.

$$\text{SPAX} + \cdot OH \rightarrow [\text{SPAX-H}] + H_2O \quad (1)$$

$$\text{SPAX} + \cdot OH \rightarrow [\text{SPAX-OH}]\cdot \quad (2)$$

$$\text{SPAX} + \cdot OH \rightarrow \text{SPAX}^{+\cdot} + H_2O \quad (3)$$

As discussions mentioned above, the transient absorption at 420 nm may be assigned to $[\text{SPAX-H}]^\cdot$, $[\text{SPAX-OH}]^\cdot$ and/or $\text{SPAX}^{+\cdot}$. According to the dependence of the observed pseudo-first order rate constants for the form of absorption at 420 nm on the various concentration of SPAX (See Fig.2), a rate constant 1.73×10^10 $dm^3 \cdot mol^{-1} \cdot s^{-1}$ for the reaction of SPAX with ·OH was obtained.

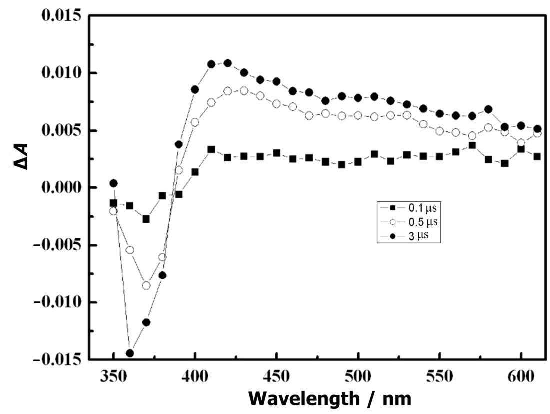

Fig.1 Transient absorption spectra recorded at different time after the pulse radiolysis of N_2O saturated 0.1 mM SPAX solution.

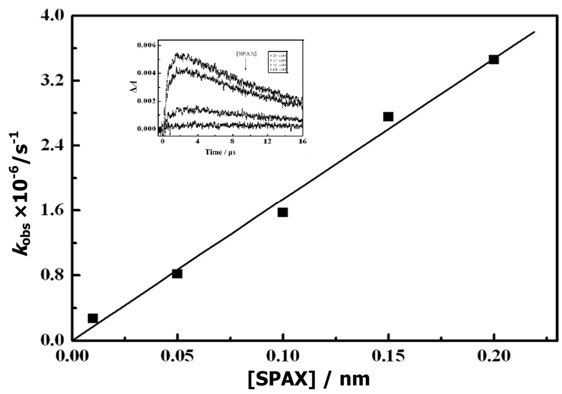

Fig.2 Dependence of the observed pseudo-first order rate constants for the form of absorption at 420 nm on the various concentration of SPAX. Inset: the time profiles at 420 nm.

3.2 One-electron oxidation reaction

To investigate the transient absorption at 420 nm deeply, the one-electron oxidation experiment was

carried out. As we know that azide radical ($\cdot N_3$) was a good electron acceptor in reactions. Hence, $\cdot N_3$ was chose as an oxidant in one-electron reaction of SPAX with $\cdot N_3$. In reaction, the concentration of NaN_3 was chose as 100 mM, which is much larger than that the concentration of SPAX, for the purpose of hydroxyl radical can reacted with azide anion firstly. As shown in Fig.3, after the pulse, a negative absorption at about 370 nm and a positive absorption at about 420 nm were observed. As discussions mentioned above, the transient absorption at 370 nm was reasonably assigned to the bleaching. According to the reaction of SPAX with $\cdot N_3$, the transient absorption at 420 nm was reasonably assigned to the SPAX radical cation ($SPAX^{+\cdot}$). The mechanism of one-electron reactions was proposed as below.

$$\cdot OH + N_3^- \rightarrow OH^- + \cdot N_3 \quad (4)$$

$$\cdot N_3 + SPAX \rightarrow N_3^- + SPAX^{+\cdot} \quad (5)$$

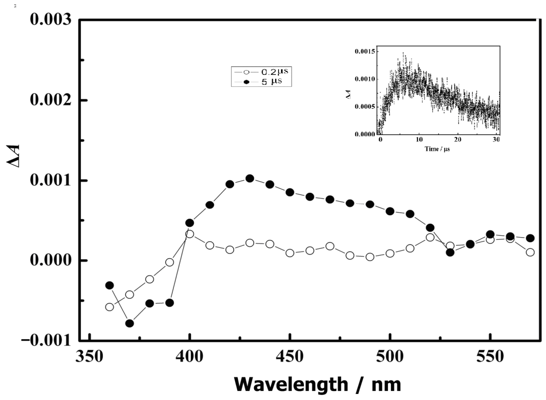

Fig.3 Transient absorption spectra recorded at different time after the pulse radiolysis of N_2O saturated 0.1 mM SPAX solution containing 0.1 M NaN_3 and 0.2 M *t*-BuOH. Inset: The time profile observed at 420 nm.

3.3 Reaction of SPAX with e_{aq}^-

To investigate the radical anion of SPAX, another pulse radiolysis experiment was carried out. In this study, the reaction of SPAX with hydrated electron (e_{aq}^-) was studied. As shown in Fig.4, at the end of pulse, the transient absorption of hydrated electron in the region of 500-700 nm was observed[26,27]. And later, another transient absorption at 410 nm appeared with the decay of the hydrated electron. As we all know, hydrated electron was the main transient product at the end of pulse. Hence, according to the reaction of one-electron reduction, the transient absorption at about 410 nm should be assigned to SPAX radical anion ($SPAX^{-\cdot}$). The transient one-electron reduction reaction of SPAX with hydrated electron was proposed as following, and the rate constant of 2.2×10^{10} $dm^3\cdot mol^{-1}\cdot s^{-1}$ was obtained according to the dependence of the observed pseudo-first order rate constants at 700 nm versus the various concentration of SPAX (Fig.4, Inset).

$$SPAX + e_{aq}^- \rightarrow SPAX^{-\cdot} \quad (6)$$

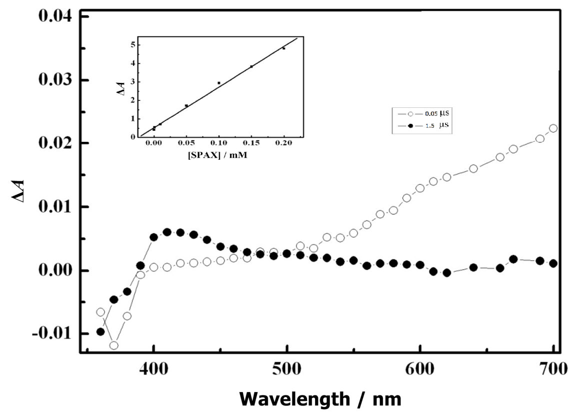

Fig.4 Transient absorption spectra recorded at different time after the pulse radiolysis of N_2 saturated 0.1 mM SPAX solution containing 0.2 M *t*-BuOH. Inset: The dependence of the observed pseudo-first order rate constants at 700 nm vs the concentration of SPAX.

4 Conclusion

The radical cation and radical anion of SPAX was investigated in this study. The transient spectra of SPAX radical cation and anion were obtained. According to the discussion, reactions of SPAX with $\cdot OH$, $\cdot N_3$ and e_{aq}^- were proposed. The rate constants for reactions of SPAX with $\cdot OH$ and e_{aq}^- were obtained as 1.7×10^{10} $dm^3\cdot mol^{-1}\cdot s^{-1}$ and 2.2×10^{10} $dm^3\cdot mol^{-1}\cdot s^{-1}$, respectively.

The results obtained in this study may be a good help for exploring the phototoxicity and DNA damage induced by SPAX.

References

1 Domagala J M, Hanna L D, Heifetz C L, *et al.* J Med Chem, 1986, **29:** 394–404.

2 Chaudhry A Z, Knapp C C, Sierramadero J, *et al.* Antimicrob Agents Ch, 1990, **34:** 1843–1845.

3 Cooper M A, Andrews J M, Ashby J P, *et al.* J Antimicrob Chemoth,1990, **26:** 667–676.

4 Doebbeling B N, Pfaller M A, Bale M J, *et al.* Eur J Clin Microbiol, 1990, **9:** 298–301.

5 Edelstein P H, Edelstein M A C, Weidenfeld J, *et al.* Antimicrob Agents Ch, 1990, **34:** 2122–2127.

6 Miyamoto T, Matsumoto J, Chiba K, *et al.* J Med Chem, 1990, **33:** 1645–1656.

7 Rolston K V I, Nguyen H, Messer M, *et al.* Antimicrob Agents Ch, 1990, **34:** 2263–2266.

8 Ji B, Truffotpernot C, Grosset J. Tubercle, 1991, **72:** 181–186.

9 Tokura Y, Iwamoto Y, Mizutani K, *et al.* Arch Dermatol Res, 1996, **288:** 45–50.

10 Smith M A, D'Aversa G, Sable M, *et al.* J Toxicol-Cutan Ocul, 1999, **18:** 65–73.

11 Pierfitte C, Royer R J, Moore N, *et al.* Brit J Clin Pharmaco, 2000, **49:** 609–612.

12 Stahlmann R, Zippel U, Forster C, *et al.* Antimicrob Agents Ch, 1998, **42:** 1470–1475.

13 Hamanaka H, Mizutani H, Asahig K, *et al.* J Dermatol Sci, 1999, **21:** 27–33.

14 Struwe M, Greulich K O, Perentes E, *et al.* J Invest Dermatol, 2009, **129:** 699–704.

15 Friedberg E C. Nature. 2003, **421:** 436–440.

16 Sanchez G, Hidalgo M E, Vivanco J M, *et al.* Photochem Photobiol, 2005, **81:** 819–822.

17 Gurbay A, Gonthier B, Signorini-Allibe N, *et al.* Neurotoxicology, 2006, **27:** 6–10.

18 Shirpoor A, Minassian S, Salami S, *et al.* Cell Physiol Biochem, 2008, **22:** 769–776.

19 Kumar S, Budhwar R, Nigam A, *et al.* Mutagenesis,2009, **24:** 495–500.

20 Liu Y C, Zhang P, Li H X, *et al.* Photochem Photobiol, 2012, **88:** 639–644.

21 Spratt T E, Schultz S S, Levy D E, *et al.* Chem Res Toxicol, 1999, **12:** 809–815.

22 Taba H, Kusano N. Antimicrob Agents Ch,1998, **42:** 2193–2196.

23 Stein G E, Havlichek D H. Pharmacotherapy, 1997, **17:** 1139–1147.

24 Shimada J, Nogita T, Ishibashi Y. Clin Pharmacokinet, 1993, **25:** 358–369.

25 Miao J L, Wang W F, Pan J X, *et al.* Radiat Phys Chem, 2001, **60:** 163-168.

26 Lu C Y, Lin W Z, Wang W F, *et al.* Radiat Phys Chem, 2000, **59:** 61–66.

27 Zhang P, Yao S D, Li H X, *et al.* Radiat Phys Chem, 2011, **80:** 548–553.

28 Morimoto S, Ito T, Fujita S I, *et al.* Chem Phys Lett, 2008, **461:** 300–304.

29 Kowalczuk D, Hopkala H, Pietras R. Chem Anal-Warsaw. 2004, **49:** 201–211.

30 Navaratnam S, Parsons B J. J Chem Soc Faraday T, 1998, **94:** 2577–2581.

31 Burrows H D, Miguel M D, Monkman A P, *et al.* J Chem Phys. 2000, **112:** 3082–3089.